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Essential oil composition, phenolic content, antioxidant, and antimicrobial activity of cultivated *Satureja rechingeri* Jamzad at different phenological stages

Abstract: *Satureja rechingeri* is a rare endemic and endangered species found in Iran. Its propagation, variations in essential oil and phenolic content, as well as antioxidant and antimicrobial activities at different phenological stages are reported in this study. The chemical composition of essential oils obtained by hydro-distillation from the aerial parts were determined by GC and GC-MS. A total of 47 compounds were identified in the essential oils of *S. rechingeri* at different phenological stages. The major components of all oils were carvacrol (83.6%–90.4%), *p*-cymene (0.8%–2.9%) and γ-terpinene (0.6%–2.4%). The total phenolic content and the antioxidant activity of methanolic extracts were determined with the Folin-Ciocalteau reagent and by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and ferric reducing antioxidant power (FRAP) assay, respectively. Total phenols varied from 35.5 to 37.5 mg gallic acid equivalents/g dry weight (dw), and IC_{50} values in the radical scavenging assay ranged from 46.2 to 50.2 mg/mL, while those in the FRAP assay were between 49.6 and 52.5 μM quercetin equivalents/g dw. By the disc diffusion method and by determination of the minimum inhibitory concentrations (MIC), the essentials oils of the various phenological stages were found to have high activities against four medically important pathogens.

Keywords: antimicrobial activity; phenological stages; *Satureja rechingeri*; secondary metabolites.

DOI 10.1515/znc-2014-4121
Received July 5, 2014; revised March 30, 2015; accepted March 31, 2015

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1 Introduction

In recent years, the essential oils and various extracts of medicinal and aromatic plants have gained great interest among researchers as sources of various natural products [1]. In this regard, the antimicrobial and antioxidant properties of these plants' secondary metabolites have formed the basis of many applications, including raw and processed preservation, pharmaceuticals, alternative medicine, and natural therapies [2, 3].

The genus *Satureja* (Lamiaceae) constitutes about 200 species of herbs and shrubs, often aromatic, which are widely distributed in Mediterranean areas, Asia, and boreal America [4]. This genus is represented in the flora of Iran by 16 species, of which ten are considered endemic (*Satureja atropatana* Bunge. *Satureja bachtiarica* Bunge, *Satureja edmondi* Briquet, *Satureja intermedia* C.A.Mey, *Satureja isophylla* Rech., *Satureja kallarica* Jamzad, *Satureja khuzistanica* Jamzad, *S. macrosiphonia* Bornm., *Satureja sahendica* Bornm., and *Satureja rechingeri* Jamzad). *Satureja rechingeri* is a rare and endemic *Satureja* species from Iran. This plant is a perennial and bushy aromatic herb that grows 50 cm high with yellow colored flowers, dense white villous hairs, and a dense covering of punctuate glands on both leaf surfaces. These plants grow in the rock walls and stony hillsides of Ilam province located in West Iran. Flowering occurs in autumn at late September and October [5].

The *Satureja* species contain some secondary metabolites, such as volatile oils, phenolic compounds, tannins, sugars, and fatty acids [6]. The aerial parts of some *Satureja* plants have been widely used in foods and herbal tea, as flavor components, and in folk and traditional medicine to treat various ailments, such as cramps, muscle pains, nausea, indigestion, diarrhea, and infectious diseases [7–9]. A previous literature review on essential oil composition in *Satureja* species showed that these are rich in terpenoids, such as carvacrol, γ-terpinene, thymol, *p*-cymene, β-caryophyllene, linalool, and other terpenoids. However, the chemical composition and

the amount of components vary among and within the *Satureja* essential oils [10].

Essential oil constituents and secondary metabolites in medicinal and aromatic plants are strongly influenced by their origin, environmental conditions, ontogenetic variation [11, 12], management practices (e.g., harvest time), and ecological and climatic conditions [13]. Therefore, antimicrobial, antioxidant, and other biological activities may vary, based on the variations in chemical compositions of essential oils and the extracts of medicinal plants [14–16].

Endemism and limitation of the natural habitats in Iran, use for medicinal purposes, and increasing collection all lead to the reduction of the *S. rechingeri* populations in their natural habitats in Iran. The current study focuses on the possibility of *S. rechingeri* propagation and an investigation into the essential oil composition, phenolic content, as well as the antioxidant and antimicrobial activities of this plant at different phenological stages. As far as we know, this is the first report on these properties of *S. rechingeri*. The results of this study can provide useful information about the propagation of this medicinal plant, and determine the suitable harvest time for obtaining the maximal amount of essential oil and secondary metabolites.

2 Materials and methods

2.1 Plant material

The cuttings of *S. rechingeri* were collected from plants grown in the wild in their natural habitat in Ilam province (between Mehran and Dehloran) in West Iran. A voucher specimen was deposited at the herbarium of medicinal and aromatic plants of Islamic Azad University, Estahban branch. The basal 2 cm of the cuttings were dipped into rooting powder containing indole butyric acid (IBA) to stimulate rooting. After hormone treatment, the cuttings were placed in a rooting medium containing sterilized river sand and then sprayed with water mist under greenhouse conditions. After about 3 months, the rooted cuttings were transferred to the Medicinal and Aromatic Plants Experimental Garden (MAPEG) of the Estahban branch, Islamic Azad University in Fars province in southwest Iran. Six months after transfer of the cuttings to the garden, the aerial parts of *S. rechingeri* were harvested at different phenological stages [pre-flowering (July), full-flowering (October), and post-flowering in seed set (November)]. The harvested plants in the three different phenological stages were dried at room temperature (25 °C) for 2 weeks, after which they were ground and powdered for essential oil extraction and further manipulation.

2.2 Essential oil isolation procedure

The powders (100 g) obtained above were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus according to the method recommended in the British Pharmacopoeia [17]. The oils were dried over anhydrous sodium sulfate, weighed, and stored in tightly closed dark vials at 4 °C prior to analysis and antimicrobial tests.

2.3 Gas chromatography (GC)

GC analysis was performed with an Agilent Technologies gas chromatograph (Series II, Model 6990; Santa Clara, CA, USA) equipped with a flame ionization detector and an HP capillary column (30 m×0.25 mm, 0.25 μm film thickness). The chromatographic conditions were as follows: the oven temperature increased from 60 °C to 240 °C at a rate of 3 °C/min; the injector and detector temperatures were 240 °C and 250 °C, respectively; and helium was used as the carrier gas at a linear velocity of 32 cm/s. The samples were injected using the split sampling technique by a ratio of 1:20. The percentage compositions were obtained from electronic integration of peak areas without the use of correction factors.

2.4 Gas chromatography-mass spectrometry (GC-MS)

The analyses of the volatile compounds were carried out on a Hewlett-Packard GC-MS system (GC 6990 Series II, MSD 7890A; Palo Alto, CA, USA) operating at 70 eV ionization energy, equipped with an HP-5 capillary column with phenyl methyl siloxane (30 m×0.25 mm, 0.25 μm film thickness) with helium as the carrier gas and a split ratio of 1:20. The retention indices for all the components were determined according to the Van Den Doll method using *n*-alkanes as standard [18]. The compounds were identified by comparison of their retention indices (RRI-HP-5) with those reported in the literature and by comparison of their mass spectra with those in the Wiley and Mass Finder 3 libraries or with published mass spectra [19].

2.5 Preparation of methanolic extract

Ground shade-dried plant material (7.5 g) was defatted with petroleum ether for 3 h and then extracted twice for 24 h with 200 mL of 90% (v/v) aqueous methanol at room temperature. After filtration through Whatman filter paper (Whatman, Little Chalfont, UK), supernatants were combined and the solvent was evaporated to a volume of about 1 mL using a rotary evaporator. The concentrated extracts were freeze-dried and weighed for yield determination. The samples were stored for further experiments.

2.6 Total phenolic content

The total phenolic content was determined with the Folin-Ciocalteu reagent as described previously [20]. Briefly, 200 μL of plant extract dissolved in methanol (1 mg/mL) were mixed with 2.5 mL of Folin-Ciocalteu reagent (diluted 10 times in distilled water) in glass tubes in triplicate. The samples were incubated at room temperature for 5 min and vortex-mixed at least twice. Then, 2 mL of 7.5% (w/v) Na_2CO_3 solution were added, and the glass tubes were incubated in the dark for

90 min with continuous shaking. The absorbance of samples was measured at 765 nm using a spectrophotometer (UV/VIS double beam PC.UVD.2980; Labomed, Los Angeles, CA, USA). Distilled water was used as blank. Different concentrations of gallic acid in methanol were tested in parallel to obtain a standard curve. Total phenolic contents were determined as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g dw).

2.7 Free radical scavenging capacity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined by the method of Brand-Williams et al. [21]. Briefly, four different concentrations of the plant extract dissolved in methanol were incubated with a 100-μM solution of DPPH in methanol in a total volume of 4 mL. After 30 min of incubation at room temperature, the absorbance was recorded at 517 nm. Methanol was used as blank, and all measurements were carried out in triplicate. Quercetin was used as reference compound. All solutions were prepared daily. The percent inhibition of DPPH free radical was calculated using the following formula:

$$\text{Percentage inhibition}\,(\%\text{I}) = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100,$$

where A_{blank} is the absorbance of the control reaction (DPPH alone), and A_{sample} is the absorbance of DPPH solution in the presence of the plant extract. The IC_{50} values denote the concentration of the sample required to scavenge 50% of DPPH free radicals.

2.8 Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed as described previously by Benzie and Strain [22] and Firuzi et al. [23]. Briefly, the fresh FRAP solution was prepared by mixing 10 mL of acetate buffer (300 mM, pH 3.6), 1 mL of ferric chloride hexahydrate (20 mM) dissolved in distilled water, and 1 mL of 2,4,6-tripyridyl-*s*-triazine (TPTZ) (10 mM) dissolved in 40 mM HCl. Plant extract dissolved in methanol (40 μL) at a concentration of 1 mg/mL was mixed with 4 mL of the FRAP solution. Absorbance was determined at 595 nm after 6 min of incubation at room temperature. Quercetin was tested at the final concentration of 10 μM and used as the reference compound. FRAP values, expressed as μmol quercetin equivalents per gram dry weight of plant material (μmol QE/g dw), were calculated according to the following formula:

$$\text{FRAP value} = (\Delta\text{AS} / \Delta\text{AQ}) \times \text{Y} \times 1000,$$

where ΔAS and ΔAQ are absorbance changes of the FRAP solution in the presence of the sample plant extract and quercetin, respectively, and Y is the extraction yield.

2.9 Microorganisms

Standard strains of *Candida albicans* (ATCC 10231), the Gram-positive bacteria *Staphylococcus aureus* (ATCC 6538) and *Staphylococcus epidermidis* (ATCC 1435), and the Gram-negative bacterium *Escherichia coli* (ATCC 25922) were all obtained from the Iranian Research Organization for Science and Technology.

2.10 Determination of antimicrobial activity by the disk diffusion method

In vitro antimicrobial activities of the essential oils of *S. rechingeri* were evaluated by the disk diffusion method, with determination of inhibition zones (IZ), using Mueller-Hinton agar for bacteria (MHA) and Sabouraud dextrose agar (SDA) for fungi [24]. Disks containing 10 μL of essential oil were used, and diameters of inhibition zones were measured in millimeters after 24 h and 48 h of incubation at 37 °C and 24 °C for bacteria and fungi, respectively. All studies were performed in triplicate. Blank disks containing 10 μL DMSO were used as negative controls. Oxacillin (1 μg/disk), tetracycline (30 μg/disk), amoxicillin (10 μg/disk), ketoconazole (20 μg/disk), and gentamicine (30 μg/disk) were used as positive reference standards to determine the sensitivity of the microorganisms. A broth micro-dilution method was used to determine the minimum inhibitory concentration (MIC) according to the National Committee for Clinical Laboratory Standards [25]. A serial double dilution of the oil was prepared in a 96-well micro-titer plate over the range of 0.02–50.00 μL/mL. The MIC is defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate visible growth. All determinations were performed in triplicate.

2.11 Statistical analysis

All data were expressed as mean±standard deviation (SD). Analysis of variance (ANOVE) was performed using the SAS software (version 9.2 for Windows). Significant differences between means were determined by Duncan's new multiple-range test. Differences between means were considered significant at the level of $p<0.05$. Correlation analyses of antioxidant activity vs. the total phenolic content were carried out using the Microsoft Office Excel program. The IC_{50} values of the antioxidant activities were calculated by the software Curve Expert (version 1.3 for Windows).

3 Results and discussion

3.1 Essential oil yield and composition

The yield and chemical composition of the essential oil of *S. rechingeri* and the retention indices are presented in Table 1. The essential oil obtained by hydrodistillation of the aerial parts of *S. rechingeri*, at different phenological stages, was found to be a yellow liquid, with yields ranging from 3.84% to 4.65% (w/w), based on dry weight. The highest and lowest oil yields were obtained in the full-flowering and pre-flowering stages, respectively. The comparison between our result and essential oil yields in all *Satureja* species showed that *S. rechingeri* had the highest oil yield among all *Satureja* species grown wild or cultivated in the world [10]. A total of 47 compounds were identified in the essential oils of *S. rechingeri* at different phenological stages. Oxygenated monoterpenes represented the main portion

Table 1: Essential oil compositions of *S. rechingeri* at different phenological stages.

No	Compound	RI[a]	Pre-flowering, %	Full-flowering, %	Post-flowering, %
1	α-Thujene	925	0.12±0.02	0.04±0.02	0.03±0.01
2	α-Pinene	932	0.07±0.01	0.05±0.02	0.03±0.01
3	Camphene	947	0.03±0.01	0.05±0.02	0.02±0.001
4	Sabinene	971	0.05±0.02	0.12±0.03	0.06±0.02
5	β-Pinene	975	0.04±0.01	0.02±0.01	0.03±0.01
6	3-Octanone	984	0.05±0.02	0.02±0.01	0.03±0.01
7	Myrcene	989	0.15±0.04	0.25±0.06	0.28±0.05
8	3-Octanonol	993	0.03±0.01	0.01±0.001	0.02±0.01
9	*n*-Decane	998	0.02±0.001	0.02±0.01	0.03±0.01
10	α-Phellandrene	1004	0.05±0.01	0.04±0.02	0.03±0.01
11	δ-3-Carene	1010	0.03±0.01	0.02±0.001	0.03±0.01
12	α-Terpinene	1015	0.44±0.11	0.21±0.07	0.36±0.09
13	*p*-Cymene	1023	2.85± 0.45	0.78±0.12	1.67±0.12
14	Limonene	1026	0.02±0.01	0.03±0.01	0.04±0.01
15	β-Phellandrene	1027	0.11±0.03	0.05±0.01	0.05±0.02
16	1,8-Cineole	1029	0.12±0.03	0.03±0.01	0.27±0.06
17	Benzene acetaldehyde	1041	0.01±0.001	0.01±0.001	0.03±0.01
18	(*E*)-β-Ocimene	1045	0.01±0.001	0.01±0.001	0.02±0.01
19	γ-Terpinene	1056	2.43±0.35	0.56±0.11	0.88±0.13
20	*cis*-Sabinene hydrate	1065	0.22±0.09	0.12±0.03	0.18±0.04
21	Terpinolene	1087	0.17±0.05	0.07±0.02	0.14±0.03
22	Linalool	1099	1.44±0.23	1.07±0.14	1.28±0.25
23	*n*-Nonanal	1103	0.08±0.02	0.16±0.05	0.27±0.08
24	Borneol	1164	0.14±0.03	0.11±0.03	0.08±0.02
25	Terpinene-4-ol	1176	1.34±0.25	1.04±0.14	1.12±0.33
26	α-Terpineol	1188	0.15±0.03	0.05±0.02	0.04±0.01
27	*n*-Dodecane	1197	0.09±0.02	0.16±0.03	0.23±0.08
28	Carvacrol methyl ether	1242	0.17±0.03	0.27±0.06	0.32±0.08
29	Thymol	1292	1.12±0.21	0.15±0.04	0.18±0.04
30	Carvacrol	1305	83.58±2.25	90.35±3.43	88.25±2.43
31	α-Terpinyl acetate	1347	0.65±0.14	0.43±0.11	0.23±0.08
32	Eugenol	1360	0.23±0.08	0.18±0.06	0.14±0.03
33	Carvacrol acetate	1375	0.11±0.03	0.04±0.02	0.14±0.04
34	*n*-Tetradecane	1399	0.06±0.02	0.04±0.02	0.08±0.02
35	(*Z*)-Caryophyllene	1406	0.03±0.01	0.01±0.001	0.04±0.02
36	(*E*)-Caryophyllene	1420	0.88±0.12	1.64±0.26	1.18±0.24
37	*trans*-α-Bergamotene	1435	0.05±0.01	0.04±0.01	0.06±0.02
38	α-Humulene	1452	0.27±0.05	0.17±0.03	0.19±0.06
39	(*E*)-β-Farnesene	1456	0.03±0.01	0.01±0.001	0.02±0.001
40	β-Bisabolene	1509	1.23±0.18	0.85±0.12	0.75±0.14
41	(*E*)-γ-Bisabolene	1537	0.41±0.07	0.23±0.07	0.33±0.10
42	Caryophyllene oxide	1581	0.33±0.06	0.25±0.05	0.15±0.04
43	n-Hexadecane	1598	0.12±0.02	0.05±0.02	0.04±0.02
44	α-Bisabolol	1682	0.04±0.02	0.06±0.02	0.08±0.03
45	*n*-Octadecane	1798	0.03±0.01	0.04±0.01	0.05±0.02
46	6,10,14-Trimethyl-2-pentadecanone	1841	0.23±0.04	0.11±0.02	0.12±0.03
47	*n*-Hexadecanoic acid	1963	0.18±0.03	0.24±0.04	0.18±0.04
	Oil yield (%w/w)		3.84	4.65	4.12
	Total		99.60	99.03	99.45

[a]RI, retention indices in elution order from an HP-5 column. Each value in the table was obtained by calculating the average of three experiments±standard deviation. Data are expressed as percentages of the total values.

of all samples (Table 1). The major components of all the oils were found to be carvacrol (83.58%–90.35%), *p*-cymene (0.78%–2.85%), and γ-terpinene (0.56%–2.43%). Sefidkon et al. [26] reported that carvacrol (84%–89.3%), γ-terpinene (2.2%–2.3%), *p*-cymene (0.6%–2.4%), and limonene (0.2%–2.6%) were the main components of *S. rechingeri* essential oil, which were obtained by different distillation methods at full flowering stage.

Table 2: Relative contents of phenolic terpenoids (carvacrol and thymol) and their precursors in the essential oils of *S. rechingeri* at different phenological stages.

Harvesting times	Carvacrol, %	Thymol, %	*p*-Cymene, %	γ-Terpinene, %	Total, %
Pre- flowering	83.58[c]	1.12[a]	2.58[a]	2.43[a]	89.71[a]
Full-flowering	90.35[a]	0.15[b]	0.78[c]	0.56[c]	91.84[a]
Post-flowering	88.25[b]	0.18[b]	1.67[b]	0.88[b]	90.98[a]

[a]Data are expressed as mg of gallic acid equivalents per g dry weight (dw). [b]Data are expressed as μg per milliliter. Lower IC_{50} values indicate the highest radical scavenging activity. Means with different letters were significantly different at the level of $p<0.05$. [c]Data are expressed as (μmol quercetin equivalent/g dry weight) (DW). Each value in the table was obtained by calculating the average of three experiments. Values followed by the same letter in a vertical row under the same row, are not significantly different ($p>0.05$). Data are expressed as percentages of the total values.

The highest carvacrol content (90.35%) was observed in the full-flowering stage and the lowest carvacrol content was observed in the pre-flowering stage (83.85%). At the full-flowering stage, the highest contents of phenolic compounds (carvacrol and thymol) and the lowest contents of their biosynthetic precursors (*p*-cymene and γ-terpinene) were observed, respectively, while the opposite was observed for the pre-flowering state (Table 2). The amounts of total carvacrol, thymol, and their precursors *p*-cymene and γ-terpinene, were the same and did not have significant differences at the three phenological stages (Table 2). Mikio and Taeko [27] and Yamaura et al. [28] proposed that the pathway of thymol and carvacrol biosynthesis includes γ-terpinene as the component involved in the aromatization process, resulting in the formation of *p*-cymene, the precursor of the oxygenated derivatives, thymol or carvacrol (Figure 1). It may be assumed that the sequence in this process is as follows: γ-terpinene, *p*-cymene, thymol or carvacrol. Our results are in agreement with those of Ozguven and Tansi [29] for *Thymus vulgaris* and Nejad Ebrahimi et al. [30] for *Thymus carmanicus*. These authors observed the maximum content of thymol, the major phenolic content, in the flowering stage, which then decreased in the post-flowering and seed set stages.

3.2 Total phenolic content and antioxidant activity

The total phenolic content of the methanolic extracts of *S. rechingeri* harvested at different phenological stages was measured by the Folin-Ciocalteau reagent and expressed as GAE. According to Table 3, the total phenolic content increased slightly, but significantly, from the pre-flowering to the flowering stage, and then decreased slightly in the post-flowering stage, albeit not significantly. Thus, the total phenolic content is highest at the flowering and post-flowering stages.

The antioxidant activities of the extracts of *S. rechingeri* were assessed by the DPPH free radical scavenging and FRAP methods. The DPPH assay determines the scavenging of stable radical species of DPPH by antioxidants

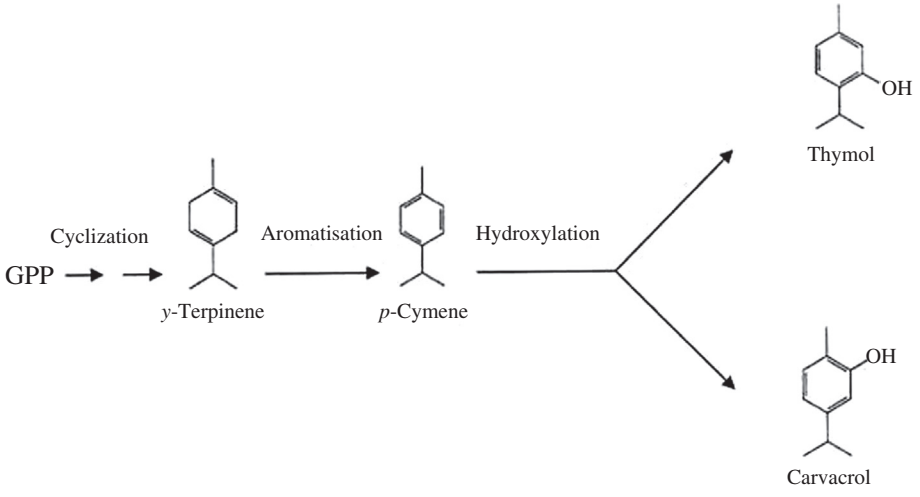

Figure 1: Thymol and carvacrol biosynthetic pathway according to Mikio and Taeko (1962). GPP: geranylgeranyl pyrophosphate.

Table 3: Total phenolic content, radical scavenging activities, and FRAP values of methanolic extracts of *S. rechingeri* at different phenological stages.

Harvesting time	Total phenolic content[a], mg GAE/g dw	Radical scavenging activity IC_{50}[b], µg/mL	FRAP value[c], µmol QE/g dw
Pre-flowering	35.52±0.43[b]	50.24±0.09[d]	49.63±0.67[b]
Full-flowering	37.48±0.58[a]	46.22±0.23[b]	52.45±0.58[a]
Post-flowering	36.23±0.23[a,b]	48.33±0.45[c]	51.33±0.25[a]
Quercetin	ND	36.54±0.45	ND

[a]Data are expressed as mg of gallic acid equivalents per g dry weight (dw). [b]Data are expressed as µg per milliliter. Lower IC_{50} values indicate the highest radical scavenging activity. Means with different letters were significantly different at the level of $p<0.05$. [c]Data are expressed as (µmol quercetin equivalent/g dry weight) (DW). Each value in the table was obtained by calculating the average of three experiments±standard deviation. Means with different letters were significantly different at the level of $p<0.05$. ND, not determined.

[31], while the FRAP assay determines the capacity for reducing Fe(III) to Fe(II) [32]. As seen in Table 3, the extract from plants at the full-flowering stage was most effective in scavenging the DPPH radical, and it also had the highest FRAP value. Thus, the two assays complemented each other; furthermore, a correlation between the antioxidant activities and the total phenolic contents was revealed. These results suggest that the major part of the antioxidant activity in *S. rechingeri* results from the phenolic compounds. This is in line with the observation of other authors who found similar correlations between the total phenolic content and the antioxidant activity of various plants [12, 16, 33–36].

3.3 Antimicrobial activity

The antimicrobial activities of *S. rechingeri* essential oils against three bacteria and one fungus were assessed by recording inhibition zones (IZ) and minimum inhibitory concentrations (MIC). *Satureja rechingeri* essential oils exhibited high antimicrobial activities against all four microorganisms tested, compared with the positive standard antibiotics (Table 4), the values of IZ and MIC being in the ranges of 61–71 mm and 0.19–0.39 mg/mL, respectively. No significant differences were observed between the antimicrobial activities of essential oils obtained at different phenological stages.

Recent studies on the essential oils of species of the Lamiaceae family have shown that most of these plants have a broad range of biological, notably antimicrobial, activities, which are generally related to the chemical composition of the oil [37]. Essential oils rich in phenolic compounds, such as carvacrol, have been widely reported to possess high levels of antimicrobial activity [38]. The antimicrobial activity of the essential oil of *S. rechingeri* can probably be attributed to high contents of their major components carvacrol, γ-terpinene, linalool, *p*-cymene, thymol, and others.

Several studies have focused on the antimicrobial activity of the essential oils and extracts of *Satureja* species, with the aim of identifying the responsible compounds [39, 40]. Carvacrol, which is the main component of *S. rechingeri* essential oil, is considered a biocide, producing bacterial membrane perturbations that lead to leakage of intracellular ATP and potassium ions and, ultimately, cell death [41, 42]. However, other components and the possible interaction between theses substances could also affect the antimicrobial activities. In fact, the

Table 4: Antimicrobial activities of the essential oil of *S. rechingeri* under different phenological stages and standard antibiotics.

Microorgaism	Harvesting times						Standard antibiotics[c]				
	Pre-flowering		Full-flowering		Post-flowering		Oxa	Tet	Amox	Keto	Gen
	IZ[a]	MIC[b]	IZ	MIC	IZ	MIC					
S. aureus	62	0.39	68	0.19	64	0.39	NA	21	17	NA	NA
S. epidermidis	61	0.39	67	0.39	64	0.39	16	28	16	17	NA
E. coli	67	0.19	71	0.19	69	0.19	NA	NA	15	NA	24
C. albicans	66	0.19	71	0.19	68	0.19	21	NA	NA	23	NA

[a]Diameter of inhibition zones (mm) including diameter of sterile disk (6 mm). Essential oil was tested at 10 µL/disk for each tested microorganism. [b]Minimum inhibitory concentration, values as mg/mL. [c]Oxa: oxacillin (1 µg/disk), Tet: tetracycline (30 µg/disk); Amox: amoxicillin (10 µg/disk); Keto: ketoconazol (20 µg/disk); Gen: gentamicine (30 µg/disk); (7–14), moderately active; (>14), highly active; NA, not active.

antimicrobial activity of essential oils may well be the result of synergy, antagonism, or additive effects of their components, which possess various potencies of activity [43]. The antimicrobial properties of the essential oil of *S. rechingeri* indicate that the plant has potential for use in aromatherapy, pharmacy, and also in pathogenic systems to prevent the growth of microbes.

Acknowledgments: The author wishes to thank the Estahban Branch of the Islamic Azad University Research and Technology Council for the financial support they provided for this work.

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